



ELSEVIER

Comparative Biochemistry and Physiology Part B 136 (2003) 833–847

CBP

www.elsevier.com/locate/cbpb

Molecular cloning and expression of the turkey leptin receptor gene[☆]

Mark P. Richards*, Stephen M. Poch

United States Department of Agriculture, Agricultural Research Service, Animal and Natural Resources Institute,
Growth Biology Laboratory, 10300 Baltimore Avenue, Building 200, Room 206, BARC-East Beltsville, MD 20705-2350, USA

Received 22 May 2003; received in revised form 13 August 2003; accepted 14 August 2003

Abstract

A cDNA encoding the long form of the turkey (*Meleagris gallopavo*) leptin receptor (LEPR) was cloned and sequenced. Turkey LEPR showed greater than 90% sequence identity at both the nucleotide and amino acid level with chicken LEPR. The LEPR gene (long form) encodes a protein of 1147 amino acids that has features similar to other LEPRs including: a signal peptide, a single transmembrane domain, and specific conserved motifs defining putative leptin-binding and signal transduction regions of the protein. In addition, a LEPR gene-related protein (LEPR-GRP) mRNA transcript was also identified and a portion of the corresponding cDNA containing the complete coding region was sequenced. The turkey LEPR-GRP gene encodes a 14-kDa (131 amino acids) protein that is distinct from LEPR. LEPR gene expression was quantified relative to β -actin in total RNA samples isolated from various tissues of 3-week-old turkey poults. Expression of LEPR was highest in brain, spleen and lung tissue with lower levels of expression in kidney, pancreas, duodenum, liver, fat and breast muscle. In developing turkey embryos, expression of LEPR was highest in brain tissue throughout incubation (days 14–28). Expression of LEPR in embryonic liver tissue peaked at day 16 and then declined toward hatching (day 28). Yolk sac expression of LEPR declined from day 14 to day 20 and then increased toward hatching. Our findings clearly demonstrate the expression of LEPR and LEPR-GRP in different tissues during embryonic and post-hatch development. In conclusion, this is the first report to identify and characterize LEPR and LEPR-GRP gene homologues in the domestic turkey.

© 2003 Elsevier Inc. All rights reserved.

Keywords: Cytokine; Development; Gene; Leptin; Receptor; Signal transduction; Turkey

1. Introduction

Leptin, a small (16 kDa) cytokine-like hormone produced primarily by adipose tissue, functions in regulating feed intake, energy balance and reproduction in mammals (Friedman, 2002). Circulating

leptin signals the brain about the status of body energy (fat) stores by binding to leptin receptors located in discrete nuclei of the hypothalamus. This, in turn, activates specific neural and neuroendocrine pathways that modulate food intake and energy expenditure in an attempt to maintain energy stores and body weight at a set level (Reidy and Weber, 2000; Friedman, 2002). Although leptin has been widely studied in mammals, less is known concerning the role of leptin in the control of feed intake and energy balance in avian species (Houseknecht et al., 1998; Reidy and Weber, 2000; Richards, 2003). A chicken leptin gene homologue

[☆] Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by USDA and does not imply its approval to the exclusion of other suitable products.

*Corresponding author. Tel.: +1-301-504-8892; fax: +1-301-504-8623.

E-mail address: richards@anri.barc.usda.gov (M.P. Richards).

has been identified and sequenced that was highly homologous (>97%) to mouse leptin (Taouis et al., 1998; Ashwell et al., 1999). However, there is considerable uncertainty concerning these reports, since other groups have been unable to verify the existence or expression of a chicken leptin gene based on the reported nucleotide sequence (Friedman-Einat et al., 1999). Thus, an investigation of the central role of the leptin system (i.e. leptin and its specific receptor) in regulating feed intake and energy metabolism in avian species is needed.

To fully understand the physiological effects of leptin, it is necessary to study the structure and function of its cognate receptor. The reported pleiotropic effects of leptin on different organ systems that regulate metabolism, reproduction, immune function, hematopoiesis, angiogenesis, bone growth and other basic physiological functions depend on signaling through the leptin receptor (LEPR, also commonly referred to as Ob-R). LEPRs have been identified for a number of domestic animal species including pig, cow, sheep and chicken (Dyer et al., 1997; Houseknecht and Portocarrero, 1998; Horev et al., 2000; Ohkubo et al., 2000; Lin et al., 2000; Ruiz-Cortes et al., 2000; Ingvarlsen and Boisclair, 2001; Chelikani et al., 2003). As a member of the class I cytokine receptor superfamily, LEPR (long form) consists of individual extracellular, membrane-spanning and intracellular (cytoplasmic) regions. The extracellular region contains multiple ligand-binding portions including: immunoglobulin (C2), cytokine receptor (CK) and fibronectin type III (F3) domains (Fong et al., 1998). The cytoplasmic region contains domains (boxes 1–3) implicated in signal transduction pathways involving Janus kinase (JAK) and signal transducers and activators of transcription (STAT), as well as, unique tyrosine phosphorylation sites (Tartaglia, 1997; Haniu et al., 1998). In general, members of the cytokine receptor family are encoded by very large genes with multiple first exons distributed over large genomic areas and are under the control of alternate upstream regulatory regions (Lindell et al., 2001).

While there has been intensive study of mammalian LEPRs, less is known about the avian LEPR gene. Sequences corresponding to LEPR long form have been reported for the chicken and expression of the LEPR gene has been demonstrated in central and peripheral tissues, including the hypothalamus (Horev et al., 2000; Ohkubo et al.,

2000; Benomar et al., 2003). Moreover, the LEPR gene has been mapped to chicken chromosome 8 (Dunn et al., 2000). Although a turkey leptin gene homologue has recently been identified and a corresponding cDNA partially sequenced (Ashwell et al., 1998), nothing is currently known concerning the function(s) of leptin or the existence of LEPR gene homologues in this avian species. Therefore, the objective of this work was to identify and begin to characterize the LEPR gene and to determine its pattern of expression in different tissues at different stages of embryonic and post-hatch development in the domestic turkey (*Meleagris gallopavo*).

2. Materials and methods

2.1. Animals

Fertile turkey eggs were incubated in a standard commercial incubator. On incubation day 14, 16, 18, 20, 22, 24, 26 and 28 (hatching), five eggs were removed and samples of embryonic liver, brain and yolk sac membrane were collected. Turkey poults were reared from hatching to 3 week of age in heated battery/brooder units. They received a standard starter poultry ration and water ad libitum. Samples from various organs were collected at 3 week of age. Tissue samples were snap frozen in liquid nitrogen and stored at -80°C prior to RNA isolation. All protocols involving the use of animals received prior approval from the Beltsville Animal Care and Use Committee.

2.2. Molecular cloning and sequencing of the turkey leptin receptor (LEPR) and leptin receptor gene-related protein (LEPR-GRP) genes

Brain total RNA and a primer-directed, reverse transcription-polymerase chain reaction (RT-PCR)-based strategy were employed to clone portions of the turkey LEPR and LEPR-GRP genes. The turkey LEPR (long form) cDNA nucleotide sequence including the complete coding region and portions of the 5'- and 3'-untranslated regions was derived using primer sets (see Table 1) initially based on sequence previously reported for chicken LEPR cDNAs (GenBank accession nos. AB033383 and AF169827). With these primers a series of overlapping PCR products was generated. The overlapping PCR products were then assembled into a single fragment of contiguous sequence.

Table 1
PCR Primers

Primer sequence (5'–3') ¹	Position ²	Orientation	Product size (bp)
ACATCAGCTGGAGGATCTGG	1–522	Forward	522
GAATAAGCATCCTCGCTTGC		Reverse	
GGAGCAATAACAACGCCAGT	460–961	Forward	502
ACAGGCTCAGACCAGCAGAT		Reverse	
TTGGTCACCCTTGATGTCAG	845–1354	Forward	510
ACTTTGCTTACGCGATCGTT		Reverse	
AGAGCGTAGCGTCCAAGAAG	1258–1754	Forward	497
TTCAAGTGTTCCCGAGCGAGT		Reverse	
TGCACATTTTCAGCCTGTTTT	1677–2185	Forward	509
ATCAGTGGCTTCCACAGGAG		Reverse	
GGCTACTGGAGCAACTGGAG	2040–2548	Forward	509
GGAACCTGCACCCACTTCAT		Reverse	
CAGCACGTGTGTGATTTTGA	2432–2928	Forward	497
TTTCCGGTTCCAGAAGAAGA		Reverse	
CGCACCGAAGAATGAAGAA	2767–3263	Forward	497
CCAGGAGCTACCTGAGCAAG		Reverse	
TCCAGATCAGGTGGGCTTTA	3153–3639	Forward	487
CTGTGGTCTCTTGACCTTG		Reverse	
GCTCAGAGGTGTGGGATTTTC	3509–3976	Forward	468
CAACACCTGGAACAAGAGCA		Reverse	

¹ Oligonucleotide PCR primer sequences designed from chicken leptin receptor sequence (GenBank Accession Nos. AF169827 and AB033383).

² Numbers corresponding to position in turkey leptin receptor sequence (GenBank Accession No. AF321982).

For LEPR-GRP, PCR primer sets were designed from EST sequence for chicken LEPR-GRP (Boardman et al., 2002). Each PCR product was subjected to bi-directional automated fluorescent sequencing using an ABI Prism 310 Genetic Analyzer with the ABI Prism big dye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA) or a Beckman Coulter CEQ 8000XL Genetic Analysis System with the dye terminator cycle sequencing (Quick Start) kit (Beckman Coulter, Inc., Fullerton, CA). Rapid amplification of cDNA ends (RACE) was employed to characterize the 3'- and 5'-ends of LEPR and LEPR-GRP cDNAs. Total RNA (1.0 µg) was used to prepare 3'- and 5'-RACE ready cDNA using the SMART RACE cDNA Amplification Kit (BD Biosciences Clontech, Palo Alto, CA). PCR was performed using Platinum Taq DNA polymerase with 3.5 mM Mg²⁺ (Invitrogen/Life Technologies, Carlsbad, CA), touchdown PCR, and LEPR and LEPR-GRP gene specific 3'-(forward) and 5'-(reverse) RACE primers. Amplified cDNA fragments and RACE products containing sequence corresponding to the

5'- and 3'-ends were either subjected to direct sequencing or were sub-cloned into the pCR[®]2.1-TOPO vector using the TOPO TA cloning kit (Invitrogen/Life Technologies) and sequenced using M13 forward and reverse sequencing primers.

2.3. Reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was isolated using Trizol reagent according to the manufacturer's recommended procedure (Invitrogen/Life Technologies). Reverse transcription (RT) reactions (20 µl) consisted of: 1 µg total RNA, 50 units SuperScript II reverse transcriptase (Invitrogen/Life Technologies), 40 units of an RNase inhibitor (Invitrogen/Life Technologies), 0.5 mM dNTPs, and 100 ng random hexamer primers. Polymerase chain reaction (PCR) was performed in 25 µl containing: 20 mM Tris-HCl, pH 8.4, 50 mM KCl, 1.0 µl of the RT reaction, 1.0 unit of Platinum Taq DNA polymerase (Hot Start), 0.2 mM dNTPs, 2.0 mM Mg²⁺

(Invitrogen/Life Technologies), 10 pmol each of the LEPR or LEPR-GRP gene-specific primers and 5 pmol each of the β -actin primers:

1. LEPR (total)¹
forward: 5'-CAGTGTGAGCCGGTACGTTA-3'
reverse: 5'-GGAACATCTTCCCAGAGCAG-3'
2. LEPR (long form)²
forward: 5'-GGGCACAAGGTGTTGATTTT-3'
reverse: 5'-GGTAATGGAGACCTCGCTCA-3'
3. LEPR-GRP³
forward: 5'-CGGGTATCAAAGCTCTCGTG-3'
reverse: 5'-CCACTGTTCCCAGCTAAAGTC-3'
4. β -actin
forward: 5'-TGCCTGACATCAAGGAGAAG-3'
reverse: 5'-TGCCAGGGTACATTGTGGTA-3'

Thermal cycling parameters were as follows: 1 cycle 94 °C for 2 min, followed by 30–35 cycles, 94 °C for 30 s, 58–60 °C for 30 s, 72 °C for 1 min with a final extension at 72 °C for 8 min. Verification of the identity of the amplicons for LEPR (total and long form), LEPR-GRP and β -actin was accomplished following RT-PCR by direct, bi-directional sequencing of each PCR product using the automated fluorescent DNA sequencing approach described above.

2.4. Capillary electrophoresis with laser-induced fluorescence detection (CE/LIF)

Aliquots (2 μ l) of RT-PCR samples were diluted 1:100 with deionized water prior to CE-LIF. A P/ACE MDQ (Beckman Coulter, Inc.) equipped with an argon ion LIF detector was used. Capillar-

ies were 75 μ m I.D. \times 32 cm μ SIL-DNA (Agilent Technologies, Folsom, CA). Enhance™ dye (Beckman Coulter, Inc.) was added to the DNA separation buffer (Sigma, St. Louis, MO) to a final concentration of 0.5 μ g/ml. Samples were loaded by electrokinetic injection at 3.5 kV for 5 s and run in reverse polarity at 8.1 kV for 5 min. P/ACE MDQ software (Beckman Coulter, Inc.) was used to calculate peak areas for the PCR products separated by CE (Richards and Poch, 2002).

2.5. Quantitation of LEPR gene expression

The level of LEPR gene expression was determined as the ratio of integrated peak area for the LEPR PCR product relative to that of the co-amplified β -actin internal standard (Richards and Poch, 2002). Values are presented as the mean \pm S.D. of five individual determinations.

3. Results

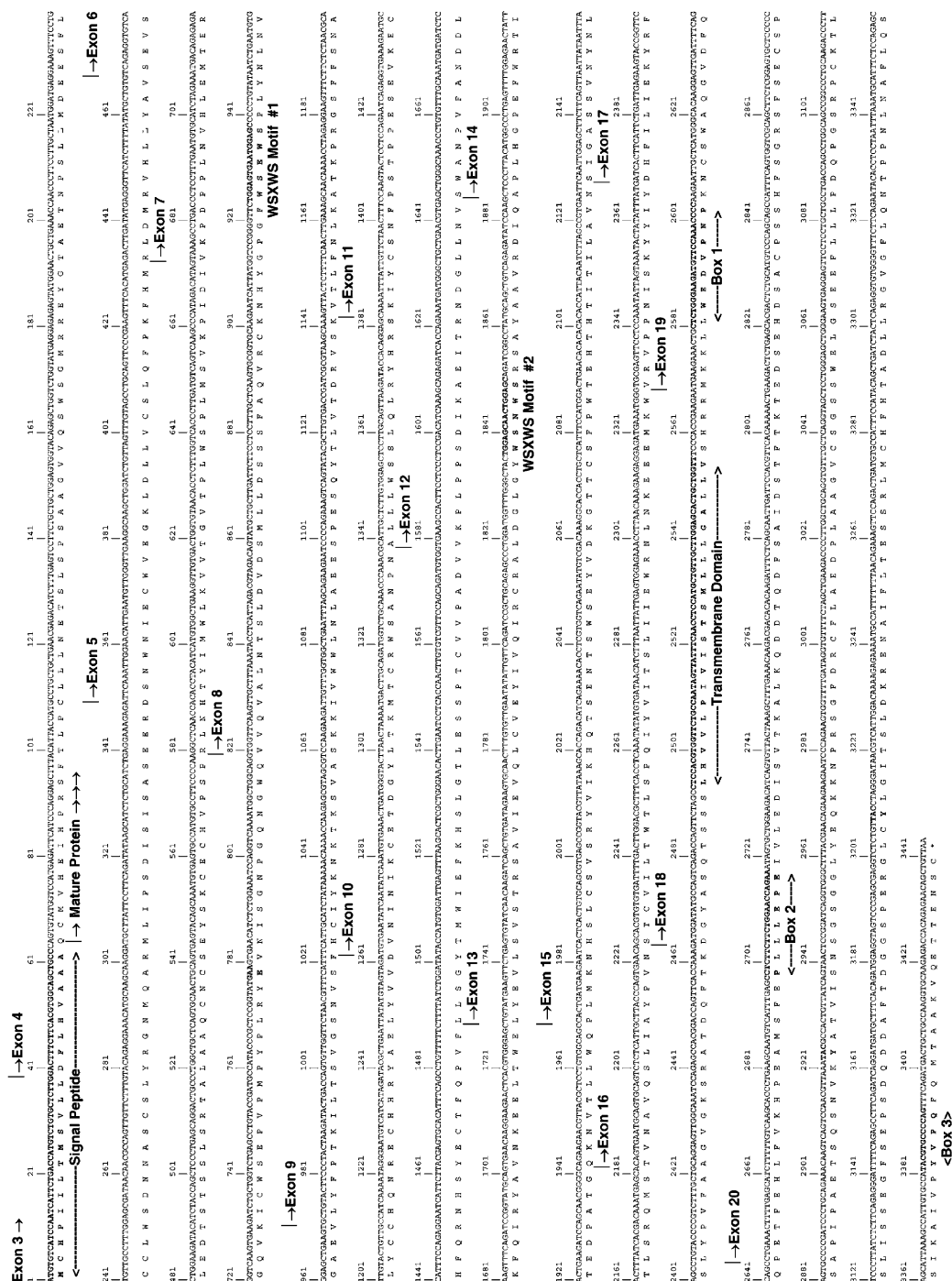
3.1. Molecular cloning and sequence analysis of turkey LEPR cDNA

A cDNA fragment derived from the LEPR gene (long form) consisting of 3976 nucleotides (nt), was cloned from turkey brain mRNA using a primer-directed, RT-PCR-based strategy and sequenced (GenBank accession number AF321982). This fragment contained an open reading frame of 3444 nt that encoded a predicted protein containing 1147 amino acids with a molecular mass of 129 kDa (Fig. 1). In addition, 209 nt of the 5'-end untranslated region (UTR) and 323 nt of the 3'-end UTR were also determined. The deduced amino acid sequence of turkey LEPR (long form) contained 38 cysteine (C) residues available for potential formation of intramolecular disulfide bonding and 56 asparagine (N) residues as potential sites for N-linked glycosylation (Fig. 1). Seventeen of the 18 cysteine residues reported to be involved in disulfide bond formation in the extracellular portion of the human LEPR were conserved in the turkey LEPR, whereas 11 of the 18 mapped N-glycosylation sites were conserved in this same region of the turkey LEPR (Haniu et al., 1998). Together, these findings suggest a similar tertiary protein structure of the turkey LEPR as compared to the human receptor.

¹ Determination of 'total' (long + short forms) LEPR was achieved by using a forward primer anchored in exon 15 and a reverse primer that annealed to a site in exon 19 (see Fig. 1). This would effectively amplify all transcripts containing sequence through the box 1 motif (see Fig. 2), that is both long and short (total cellular) forms, but not those transcripts that lack the transmembrane domain region located in exon 18 (often referred to as the 'soluble' receptor or Ob-R_c).

² The determination of long form LEPR was made possible by designing a reverse primer within the sequence corresponding to exon 20 (see Fig. 1). By doing so, only cDNA transcripts corresponding to the full-length (long form) receptor would be amplified.

³ Oligonucleotide primers for leptin receptor gene-related protein (LEPR-GRP) were designed from a chicken expressed sequence tag: EST #045306.2 (Boardman et al., 2002).



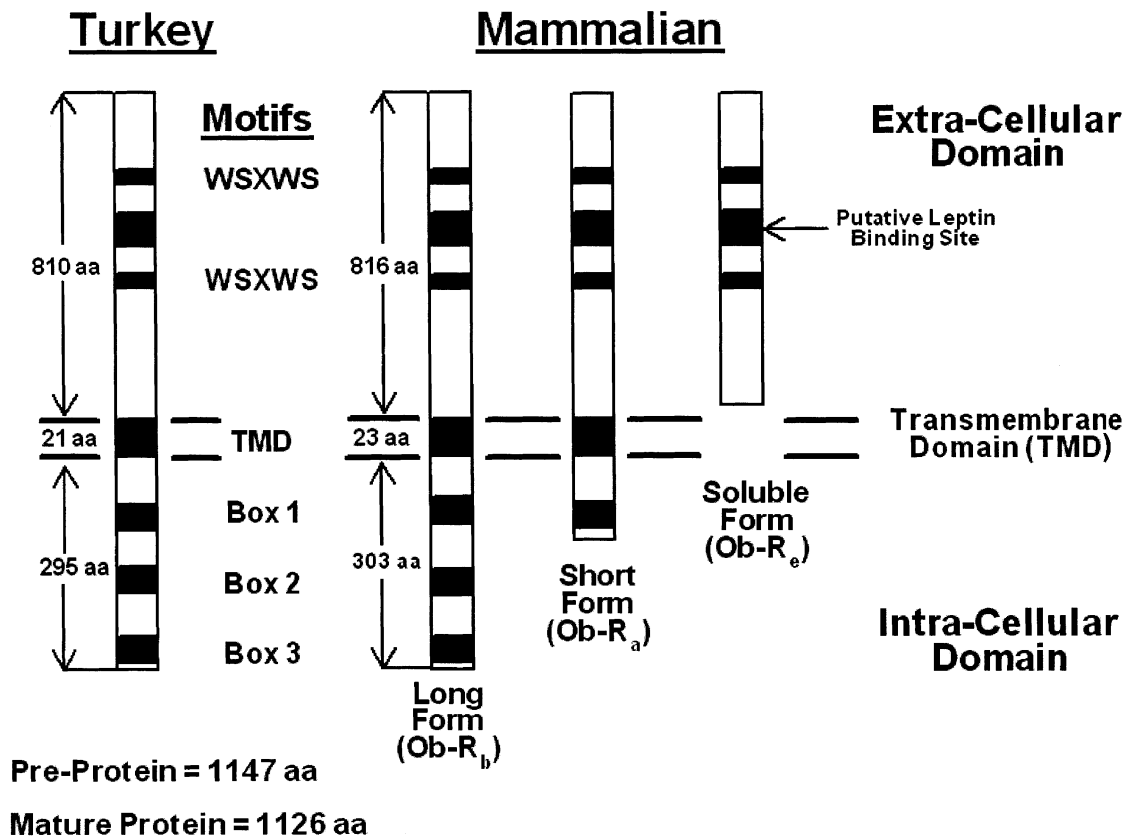


Fig. 2. A schematic representation of turkey and mammalian LEPRs (long form) detailing the overall size (in amino acids, aa) and positioning of specific conserved structural motifs and the nature of LEPR splice variants that have been identified for the mammalian receptor (Ob-R_{a-e}).

3.2. Features of the turkey LEPR

The turkey LEPR (long form) is predicted to possess a signal peptide comprised of 21 amino acids, an extracellular-domain of 810 amino acids, a single transmembrane domain of 21 amino acids, and an intracellular domain consisting of 295 amino acids (Fig. 2). A number of features characteristic of members of the class I cytokine receptor super-family were also found in the turkey LEPR (Table 2). These include the presence of multiple cytokine receptor (CK), immunoglobulin C2-like (C2) and fibronectin type III (F3) domains (Fong et al., 1998). In addition, a pair of repeated tryptophan/serine motifs (WSXWS) was found in the extra-cellular region of the turkey LEPR forming an approximate boundary for the putative leptin-binding domain that consisted of combined CK-F3 domains (see Fig. 1 and Table 2). The intracellular domain contained three conserved

regions (boxes 1–3) that have been reported to be involved in the binding of JAK2 and STAT-3, STAT-5 and STAT-6 in mammalian LEPRs (Ghiardi and Skoda, 1997). In addition, the intracellular domain contained three conserved tyrosines (Y975, Y1070 and Y1128; see Fig. 1) that are thought to serve as intracellular phosphorylation sites essential for leptin-induced signal transduction (White et al., 1997a). The extra-cellular domain contained a conservative amino acid change of glutamine to glutamic acid at position 260 (Gln260Glu). In the fatty (*fa/fa*) Zucker rat, a single base change at this site results in a point mutation (A•C at nt position 806) and causes a proline to be substituted (Gln269Pro) that deactivates the signaling capacity of the receptor resulting in the observed obese phenotype (White et al., 1997b).

The structure of the turkey LEPR cDNA is highly conserved with respect to the number, order

Table 2
Features of the turkey leptin receptor (Long Form) mRNA and protein

Feature	Nucleotide position ¹	Amino acid position ²	Description
5'-UTR	<1–209	–	5' end mRNA untranslated region
CDS	210–3653	1–1147	Coding region
Signal peptide	210–272	1–21	Signal peptide sequence
Mature protein	273–3650	22–1147	Functional receptor
Extra-cellular domain	273–2702	22–831	Extra-cellular portion that includes the leptin-binding domain
Mutation site (<i>fa/fa</i>)	987–989	260	Site of Gln-Pro substitution in the 'fatty' Zucker rat (<i>fa/fa</i>)
C2 domain	1164–1466	319–419	Immunoglobulin C2-like domain
Loop	720–881	171–224	Connecting loop
CK domain	381–719	58–170	Cytokine receptor domain
F3 domain	1467–1793 ³	420–528 ³	Fibronectin type III domain
	882–1163	225–319	
	1794–2087 ³	529–626 ³	
	2088–2375	627–722	
WSXWS motif	2376–2702	723–831	Conserved tryptophan/serine repeat
	1134–1148	309–313	
	2046–2060	613–617	
Transmembrane domain	2703–2765	832–852	Membrane spanning region
Intra-cellular domain	2766–3650	853–1147	Cytoplasmic signaling portion (long form)
Box 1 Motif	2771–2800	868–877	JAK-binding site
Box 2 Motif	2907–2907	900–906	JAK-binding site
Box 3 Motif	3591–3602	1128–1131	STAT-binding site
Conserved tyrosines	3132–3134	975	Intra-cellular receptor phosphorylation sites essential for signaling
	3417–3419	1070	
	3491–3493	1128	
3'-UTR	3654–3976	–	3' end mRNA untranslated region

¹ Numbers corresponding to position in turkey leptin receptor nucleotide sequence (GenBank Accession No. AF321982).

² Numbers corresponding to position in turkey leptin receptor amino acid sequence (GenBank Accession No. AAG40323).

³ Region corresponding to the putative leptin-binding domain.

and size of each of the 18 coding exons sequenced (Table 3). This is similar to what has been reported for the comparison of chicken to mammalian LEPR genes (Horev et al., 2000). Based on this comparison, predicted exon boundaries were completely conserved in the turkey LEPR cDNA also. Table 3 shows a size comparison of the 18 exons (exons 3–20) that comprise the coding region for LEPRs from different species. It is clear from this comparison that exon sizes are quite similar across different species and that exons 9 and 17, in particular, display an invariant size. It is interesting to note that the highly conserved exon 9 also contained a portion of the putative leptin-binding domain (Fong et al., 1998; Horev et al., 2000; Sandowski et al., 2002). Exons 4 and 20, encoding the N- and C-terminal (long form) portions of the extra-cellular and intra-cellular domain regions of LEPR, respectively, showed the highest degree of size variability.

The coding region of turkey LEPR was most similar at the nucleotide and amino acid sequence levels to chicken (>90% identity) but less so (approx. 40–60%) to mammalian LEPRs (Tables 4 and 5). Within the putative leptin-binding domain, increased nucleotide and amino acid sequence homology was observed when comparing turkey LEPR with other LEPRs (data not shown). A high degree of conservation of specific amino acid residues was also observed in the regions of turkey LEPR and leptin proteins that interact in the formation of a leptin/LEPR complex (Fig. 3a and b).

3.3. Expression of turkey LEPR in various tissues

Expression of both total (cellular) LEPR (short + long forms) and just long form LEPR was highest in whole brain tissue from 3-week old turkey poult. Lung and spleen also demonstrated

Table 3
Size comparison for exons comprising leptin receptor (long form) gene coding region for different species

Exon number	Turkey ^{1b}	Chicken ² (nucleotides)	Mouse ³	Human ⁴
3 ^a	38	38	40	40
4	311	311	330	330
5	118	118	124	124
6	205	205	206	209
7	147	150	143	146
8	151	145	145	145
9	291	291	291	291
10	121	118	118	118
11	196	196	200	200
12	150	150	149	149
13	156	156	160	160
14	84	84	83	83
15	213	213	217	217
16	186	186	183	183
17	96	96	96	96
18	105	105	106	106
19	75	75	76	76
20 ^c	801	804	822	825
Total CDS ^d	3444	3441	3489	3498

^a Based on sequence data from GenBank Accession Numbers:

¹AF321982, ²AF169827, ³U46135, ⁴NM_002303.

^a Only sequence beginning with the initiation codon is included.

^b Exon/intron junctions determined according to Horev et al. (2000).

^c Only sequence through the stop codon is included.

^d Sum of exons comprising the coding region.

high levels of LEPR expression, whereas liver had the lowest level of expression. In general, expression level relative to β -actin of the long form was less than that of total LEPR in all tissues examined, suggesting the possible existence of short form splice variants (Fig. 4).

LEPR (total) was expressed in the developing turkey embryo where it was detected in brain, liver and yolk sac tissues between day 14 of incubation and hatch (day 28). Expression of

LEPR in brain remained elevated throughout incubation, whereas, expression in liver peaked at day 16 and declined toward hatch. Yolk sac expression of LEPR declined to its lowest level on day 20 and then increased again toward hatching. Together these results suggest differential regulation of total (cellular) LEPR expression during embryonic development of the turkey (Fig. 5).

3.4. Expression of turkey LEPR-GRP in various tissues

Using PCR oligonucleotide primers designed from a chicken EST sequence for LEPR-GRP (Boardman et al., 2002), evidence was obtained for the expression of a turkey LEPR-GRP gene transcript (Fig. 6). A combination of primer-directed RT-PCR and RACE yielded sequence information for a portion of the turkey LEPR-GRP cDNA containing the entire coding region and portions of the 5'- and 3'-UTRs (data not shown). Translation of the coding region revealed a predicted protein of 131 amino acids that was distinct from the LEPR protein (Fig. 6). A species (turkey vs. human, mouse and rat) comparison of the entire coding region revealed that turkey LEPR-GRP showed much higher sequence homology both at the nucleotide (approx. 80%) and amino acid level (approx. 90%) than was observed for the same comparison of turkey LEPR (Tables 4 and 5). This suggests a higher level of conservation for the LEPR-GRP compared to the LEPR gene. Finally, a comparison by co-amplification of LEPR and LEPR-GRP gene transcripts in various tissues of 3-week old turkey poults indicated that LEPR-GRP appears to be more highly expressed than LEPR in all tissues studied (Fig. 7). Those tissues with the highest levels of LEPR expression (i.e. brain, lung, spleen) appeared to have lower levels of LEPR-GRP expression, whereas, the

Table 4
Species comparison of leptin receptor (long form) open reading frame. Percentage of nucleotide identities

	Size (nt)	Human ¹	Pig ²	Rat ³	Mouse ⁴	Chicken ⁵	Chicken ⁶	Turkey ⁷
Human ¹	3498	100	88	81	81	62	62	63
Pig ²	3498		100	80	80	62	61	62
Rat ³	3489			100	93	60	60	61
Mouse ⁴	3489				100	60	60	60
Chicken ⁵	3447					100	99	95
Chicken ⁶	3441						100	94
Turkey ⁷	3444							100

GenBank Accession Numbers: ¹NM_002303, ²AF092422, ³NM_012596, ⁴U46135, ⁵AB033383, ⁶AF169827, ⁷AF321982

Table 5

Species comparison of leptin receptor (long form) open reading frame. Percentage of amino acid identities and conservative changes

	Size (aa)	Human ¹	Pig ²	Rat ³	Mouse ⁴	Chicken ⁵	Chicken ⁶	Turkey ⁷
Human ¹	1165	100	83(89)	75(85)	75(84)	48(64)	47(63)	48(64)
Pig ²	1165		100	74(84)	74(83)	48(65)	47(64)	48(64)
Rat ³	1162			100	91(94)	47(63)	46(63)	47(63)
Mouse ⁴	1162				100	47(63)	46(62)	47(63)
Chicken ⁵	1148					100	97(97)	92(95)
Chicken ⁶	1146						100	90(93)
Turkey ⁷	1147							100

GenBank Accession Numbers: ¹NP_002294, ²AAC61766, ³NP_036728, ⁴AAC52408, ⁵BAA94292, ⁶AAF31355, ⁷AAG40323

expression of LEPR-GRP appeared to be higher in liver, a tissue that exhibited a lower level of LEPR expression as compared to brain, lung or spleen tissues.

4. Discussion

We have identified and characterized the expression of a turkey LEPR gene homologue. Based on translation of the open reading frame derived from cDNA sequencing, a 129 kDa pre-protein was predicted to consist of 1147 amino acids that showed >90% and ~60% homology at the nucleotide level with chicken and typical mammalian LEPRs, respectively. From RT-PCR analyses, we concluded that the LEPR gene was expressed in all tissues examined, including embryonic tissues.

LEPR mRNA was widely detected in tissues of the turkey with brain, lung and spleen being sites of relatively high gene expression. Such widespread expression of LEPR in peripheral and central nervous system tissues suggested a potentially important role for leptin in the development of turkeys and is consistent with the reported pleiotropic effects of this polypeptide hormone on a number of important physiological systems throughout development (Houseknecht et al., 1998; Friedman, 2002). The fact that we found significant expression of LEPR in turkey embryonic tissues emphasizes a potential role for the leptin system in avian embryonic growth and development. In fact, others have suggested the importance of the leptin/LEPR system to embryonic brain growth and development in mice and pigs (Udagawa et al., 2000; Lin et al., 2001). Moreover, high levels of expression of leptin and LEPR isoforms in the mouse placenta and fetus highlight the potential importance of the leptin system to fetal growth and development (Hoggard et al., 2000).

In mammals, LEPR gene expression is regulated by a number of mechanisms, some of which may be tissue-specific (Lee et al., 1996; Tartaglia, 1997; Chua et al., 1997; Lindell et al., 2001). Alternative splicing at the 3'-end of the gene transcript results in at least five distinct mRNA transcripts in mice (Ob-R_{a-e}) that produce a variety of LEPR protein isoforms including: (1) the complete protein with all regions intact ('b' or long form); (2) a series of proteins lacking portions of the intra-cellular region ('a', 'c', 'd' or short forms); and (3) a receptor consisting exclusively of the extra-cellular region ('e' or soluble form). The long form of LEPR has been reported to be capable of full signal transduction via the JAK/STAT pathways, whereas the truncated forms exhibit partial or no signaling capabilities (Bauman et al., 1996; Tartaglia, 1997). Because of diminished or lacking signal transduction, the truncated isoforms of LEPR have been suggested to have alternative functions such as uptake, transport or clearance of leptin from circulation (Tartaglia, 1997). However, the biological importance of the long form of LEPR in maintaining body weight and energy homeostasis has been definitively demonstrated by the resulting obese phenotype (diabetic and obese) of mice that produce only truncated forms of LEPR due to a specific genetic (*db/db*) mutation (Chen et al., 1996).

In this study, all tissues examined exhibited higher expression of total (cellular) as compared to the long form of LEPR, which could indicate the presence of additional LEPR splice variants in the turkey. Preliminary experimental results from Northern analysis indicated that two LEPR mRNAs of different size may exist in chicken hypothalamic tissue and suggested that both long and short forms of the receptor might be present in birds (Horev et al., 2000). It is interesting to note that the flanking sequence at the point of

(a)		1	11	21	31	41	51	61	71		
consensus		WSDWSTP	FTT-QDV	YFPPKILTSVGSNSV	SPHFCIYKNNQ	V	SKKIVVW	NLAEKIPESQY	V	DRVSKVTFFNLK	
Turkey		..E..	PLYNLNVGAE	L..T.....	KT.KS.A.....	L.....	ES.....	TL.T.....	L.....	
Chicken ¹		..E..	LYNLNVGAE	L..T.....	KT.S.A.....	L.....	E.....	TL.N.....	L.....	
Chicken ²		..E..	LYNLNG	-E.L..T.....	KT.S.A.....	L.....	E.....	TL.N.....	L.....	
Mouse		S.QV.....	V.....	A.....	IIS.Q.....	R.....	I.....	SI.S.....	S.....	
Rat		L.QL.....	M.....	A.C.....	TIS.Q.....	M.....	T.....	NT.S.HI.....	S.....	
Pig		FT.....	I.....	I.....	KI.S.....	M.....	Q.....	DV.G.H.....	P.MN	
Human		RV.....	I.....	K.KI.P.....	E.....	M.....	Q.....	DV.S.H.....	N
		81	91	101	111	121	131	141	151		
consensus		ATKPRGKFTYDAVYCCN-E	ECHHRYAELYVIDVNINIS	CETD	GYLT KMTCRWS	N	IQSL	GSTLQLRYHRSS	LYCSD		
Turkey		S.FSN.L...	HQNR.....	V.....	K.....	A.PNAL.LW.S.....	KI...	NF		
Chicken ¹		S.F.N.L...	HQNR.....	V.....	K.....	A.PNAL.L.S.....	KI...	NF		
Chicken ²		S.F.N.L...	HQNR.....	V.....	K.....	A.PNAL.L.S.....	KI...	NF		
Mouse		..R.....	QA.....	PST...V..V.....	R...	P.S		
Rat		..R.....	QA.....	V...I...Y.T.L.T.	P.P.....	PST...V..V.....	R...	P.N		
Pig		H.....	T.A...V.....	V		
Human		E.....	H.....	TST...AE.....	I		
		161	171	181	191	201	211	221	231		
consensus		PS	P	SE	K	C	LQRDGFYEC	FQPIFLL SGYTMWIRINHSLGSLDSPPTCV	PDSVVKPLPPSNVKAET	N	GLLKV
Turkey		..TP.E..	V.E.HF..	NHS...T..V.....	EFK...	T.E.S...V.AD.....	DI...	R.D...	N.	
Chicken ¹		..TP.E..	V.E.HF..	NHS...T..V.....	ELK...	T.E.S...V.AD.....	I...	R.D...	N.	
Chicken ²		..TP.E..	V.E.HF..	NHS...T..V.....	H...ELK...	T.E.S...V.AD.....	I...	R.D...	N.	
Mouse		..IH.T..	P.N.V.....	V.....	L.....	V.T.....		
Rat		..IR.T..	L.N.V..T.....	V.....	L.....	I.T.....		
Pig		..VH.I..	P.D.Q.....	I.....	P.....	I.....	S.....	AKI...	I	
Human		..IH.I..	P.D.Y..S.....	I.....	L.....	S.....	I.I...	I	
		241	251	261	271	281	291	301			
consensus		SWEKPVFP	PENN LQFQIRYGLNGKE	QWK	YEV	D	K	KSASL	V	DLCAVY	VQVRCRRLDGLGYWSNWS
Turkey		..AN...	ANDD.K....	AV.KE.LT.EL...	LSVSTR..	VIE.-Q..	VE.I..I..	A.....
Chicken ¹		..TN...	TNDD.K....	AV.RE.LT.EL...	LSVPTR..	VIE.-Q..	VE.I..I..	A.....
Chicken ²		..TN...	TNDD.K....	CAV.RE.LT.EL...	LSVPTR..	VIE.-Q..	VE.I..I..	A.....
Mouse		S...I...TH..	F.A.S....	L.S....	V.....
Rat		I...TH..	F.A.S....	P.S....	V.....
Pig		S...V...I...Y.T.L.T.	P.P....	A.....	K.....
Human		S...V...M...Y.A.S..	V...P.P....	A.....	K.....
(b)		1	11	21	31	41	51	61	71	81	
consensus		M	W	PLCRFLWLW	YL	YVQAVPIQ	VQDDTK	LTI KTI	IVTRINDISHTQSVSAKQRTVGLDFIPGLHPILSLSKMDQTLAVYQQ	LTSLP	
Turkey		-----	-----	..C.IF.....	-----	-----	S.....	V.....	
Chicken		..C.R.....	-----	S..V.....	C.IF.....	-----	-----	-----	V.....	
Mouse		..C.R.....	-----	S..S.....	K.....	-----	-----	-----	V.....	
Rat		..C.R.....	-----	S..S.....	HK.....	-----	R.....	-----	I.....	
Pig		..RCG.....	-----	P..S..E.....	WR.....	-----	S...M...	-----	V.....	I...I...	
Human		..H.GT..	G....P..F.....	K.....	-----	-----	S..K.....	-----	T.....	I...M.	
		91	101	111	121	131	141	151	161		
consensus		SQNVLQ	IAN DLENRDL	LHLLAF	SKSCSLPQTSGL	KPESLDGVLEASLYSTEVVALSR	LQGS	LQDILQQLD	SPEC		
Turkey		H.....	L.....	I.....		
Chicken		Q.....	I.....		
Mouse		Q.....	V.....		
Rat		H.....	R..Q.....	L.....		
Pig		..R..I..	S.....	S...P...ARA	ETL..G.....	A...M.R...	L..G.		
Human		..R..I..	S.....	V...H..WA..	ETLD..G.....	G.....	M.W...L..G.		

Fig. 3. A species comparison of the amino acid sequence of the putative leptin-binding region of LEPRs (a) and leptin proteins (b). A consensus sequence based on all species presented is also shown. Letters in bold type indicate specific amino acid residues thought to be important in formation of the leptin/LEPR complex.

divergence for short vs. long forms of LEPR (amino acid 29 of the intracellular domain located at the junction of exons 19 and 20) is conserved in chickens and turkeys as it is in mammalian LEPR counterparts (Tartaglia, 1997). This strongly

indicates that the same or similar alternative splicing mechanisms involving exon 20 (the 3' terminal exon in long form LEPR transcripts) could also be active in birds, as observed in mammals, to generate shortened forms of LEPR. However, to

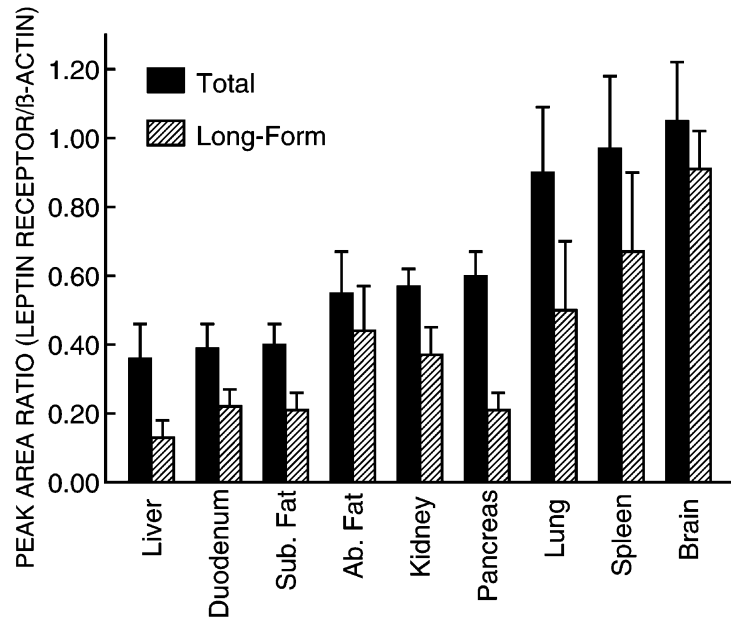


Fig. 4. Expression of LEPR (relative to β -actin) for different tissues obtained from 3-week old turkey poults. Expression of total cellular (long+short forms) and long form LEPR was determined using the specific PCR primer sets specified in the Materials and Methods section. Values represent the mean \pm S.D. of five determinations.

date, there have been no definitive reports of specific avian LEPR 3'-alternative splice variants. Characterization of alternatively spliced 3' terminal exons in reverse transcribed cDNAs would allow for the design of oligonucleotide primer sets that could be used to specifically detect and quantify

the presence of such variant gene transcripts by RT-PCR. Such a procedure was employed in this study to exclusively determine and quantify only long form LEPR transcripts by designing a reverse primer that anneals to sequence in exon 20. Thus, only those transcripts containing this 3' terminal

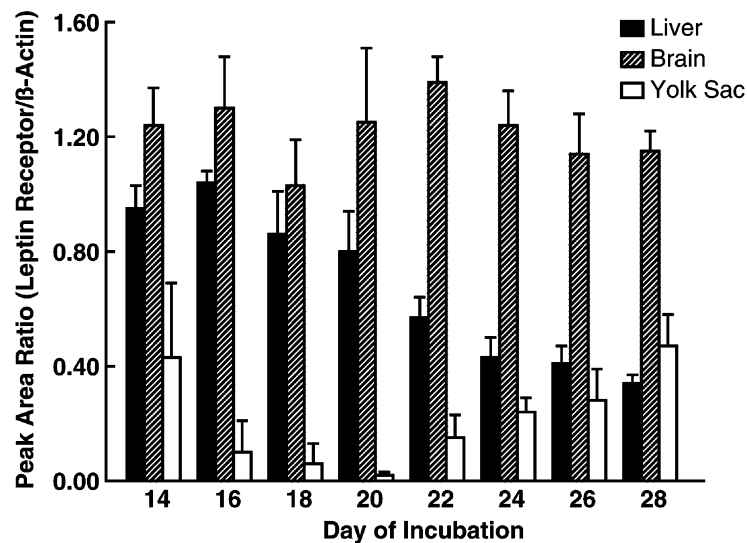


Fig. 5. Expression (relative to β -actin) of LEPR (long form) in brain, liver and yolk sac tissues obtained from turkey embryos between day 14 of incubation and hatching (day 28). Values represent the mean \pm S.D. of five determinations.



Fig. 6. Nucleotide and predicted amino acid sequence of the complete coding region for the turkey LEPR-GRP gene. The position of conserved box 1 (⁴⁶PIP⁴⁸, JAK-binding motif) is indicated by bold and underlined letters in the amino acid sequence. Also, shown (bold and underlined letters) are 3 domains corresponding to potential membrane spanning regions (transmembrane domains).

exon were detected. To date, we have not been successful in detecting any alternative 3' splicing using the RACE procedure to amplify cDNA 3'-ends. Clearly, more work is required to confirm these preliminary findings and to determine if turkey LEPR transcripts are subject to alternative splicing at their 3'-end as occurs in mammalian LEPRs.

Several receptors belonging to the cytokine class I receptor super-family such as interleukin receptors 1, 3, 11 and the growth hormone receptor exhibit differing 5'-UTRs derived from alternative promoter regulatory element and initiation codon usage (Lindell et al., 2001). In general, very large genes encode these types of receptors that contain multiple first exons distributed over large genomic regions and are controlled by alternate upstream regulatory regions (Lindell et al., 2001). In the case of mammalian LEPR genes, splicing does occur at the 5'-end of the initial gene transcript resulting in mRNAs with alternative 5'-UTRs that

may be used for tissue-specific regulation of gene expression or result in the production of a completely different protein product. One example is LEPR-GRP which results from a shift in the open reading frame due to the use of an alternative initiation codon located in the 5'-UTR of the LEPR transcript in combination with splicing of two new downstream exons (Bailleul et al., 1997; Lindell et al., 2001). The result is a transcript that codes for a 14-kDa protein completely distinct from LEPR. Another example is the novel 'overlapping' gene transcript (LEPROTL1) highly homologous to LEPR-GRP. The LEPROTL1 transcript is produced through use of a promoter shared with LEPR and an alternative initiation codon (located in the 5'-UTR of LEPR) that gives rise to a unique protein, also of 131 amino acids (14 kDa), but different from LEPR-GRP (Huang et al., 2001).

Our report of a turkey LEPR-GRP is the first report of the existence of this unique gene transcript in birds and indicates the potential for

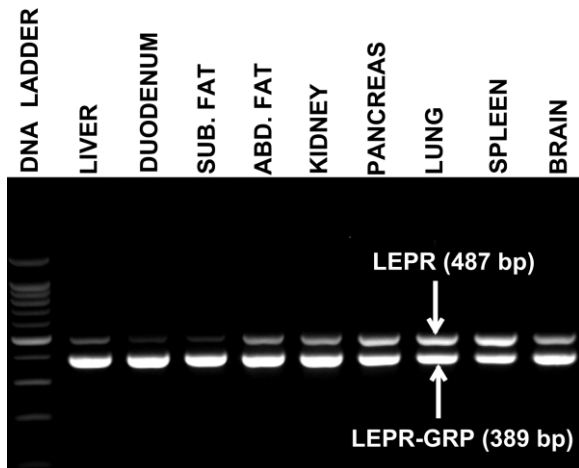


Fig. 7. Co-amplification of LEPR and LEPR-GRP in total RNA samples isolated from different tissues obtained from 3-week old turkey poult and subjected to RT-PCR. The resulting PCR products (LEPR, 487 bp amplicon; LEPR-GRP, 389 bp amplicon) were separated on a 1.5% agarose gel and stained with ethidium bromide.

alternative splicing occurring at the 5'-end of the initial avian LEPR gene transcripts and/or alternative promoter regulatory element and initiation codon usage. However, we have thus far, not been able to confirm the shared use of the first two exons for LEPR and LEPR-GRP in turkeys as has been reported in humans and rodents (Bailleul et al., 1997; Lindell et al., 2001). Moreover, the exact mechanisms responsible for the expression of the turkey LEPR-GRP homologue remain to be elucidated. The apparent inverse relationship in expression of LEPR and LEPR-GRP in a wide variety of tissues from 3-week old turkey poult might suggest the shared use of common genomic components such as promoter regulatory regions and/or alternate first exons. However, a more detailed study of the expression of these transcripts is needed to confirm the existence of such a relationship. Because of sequence homology to a yeast gene (Vps55p) involved in membrane trafficking to the vacuole, it has been suggested that LEPR-GRP may function in trafficking to the lysosome in eukaryotic cells (Belgareh-Touze et al., 2002). Our confirmation of a conserved JAK2 binding site (box 1, ⁴⁶Pro-ILE-Pro⁴⁸) and the presence of three transmembrane domains in turkey LEPR-GRP (see Fig. 6) are consistent with such a basic membrane-associated role for this putative protein in turkeys.

Molecular modeling of the leptin/LEPR complex (Fong et al., 1998; Hiroike et al., 2000; Raver et al., 2002) and information gathered from in vitro studies of leptin binding to recombinant human leptin-binding domain (Sandowski et al., 2002) should aid our understanding of how leptin signals through its receptor to control feed intake, energy metabolism and body weight. The key events leading to leptin-stimulated signaling are receptor dimerization (Raver et al., 2002) and conformational change (Couturier and Jockers, 2003) caused by binding of the ligand to the extracellular domains of two (dimerized) receptors. Moreover, studies indicating specific aspects of the structure of the leptin-binding domain and those amino acid residues crucial to leptin binding help to explain the efficacy of different recombinant leptin proteins across a range of species. For example, recombinant chicken, ovine and human leptin proteins administered via a variety of routes have proven to be efficacious in suppressing feed intake in chickens (Dridi, et al., 2000; Denbow et al., 2000; Taouis et al., 2001). However, recombinant mouse leptin was without effect in broiler chickens when administered intracerebroventricularly (Bungo et al., 1999). Clearly, understanding the structure of the avian LEPR and the mechanism of leptin signaling through this receptor will contribute to an understanding of leptin action in avian species and provide valuable insight into the role of this hormone in regulating feed intake and energy balance in birds. This is especially important in light of the uncertainty surrounding the avian leptin gene (Friedman-Einat et al., 1999).

References

- Ashwell, C.M., Czerwinski, S.M., McMurtry, J.P., 1998. Turkey leptin (ob) mRNA, partial cds. GenBank Accession Number AF082501.
- Ashwell, C.M., Czerwinski, S.M., Brocht, D.M., McMurtry, J.P., 1999. Hormonal regulation of leptin expression in broiler chickens. *Am. J. Physiol.* 276, R226–R232.
- Bailleul, B., Akerblom, I., Strosberg, A.D., 1997. The leptin receptor promoter controls expression of a second distinct protein. *Nucleic Acids Res.* 25, 2752–2758.
- Bauman, H., Morella, K.K., White, D.W., Dembski, M., Bailon, P.S., Kim, H., et al., 1996. The full-length leptin receptor has signaling capabilities of interleukin 6-type cytokine receptors. *Proc. Natl. Acad. Sci. USA* 93, 8374–8378.
- Belgareh-Touze, N., Avaro, S., Rouille, Y., Hoflack, B., Haguenaer-Tsapis, R., 2002. Yeast Vps55p, a functional homolog of human obesity receptor gene-related protein, is involved in late endosome to vacuole trafficking. *Mol. Biol. Cell* 13, 1694–1708.

- Benomar, Y., Rideau, N., Crochet, S., Derouet, M., Taouis, M., 2003. Leptin fully suppresses acetylcholine-induced insulin secretion and is reversed by tolbutamide in isolated perfused chicken pancreas. *Horm. Metab. Res.* 35, 81–85.
- Boardman, P.E., Sanz-Ezquerro, J., Overton, I.M., Burt, D.W., Bosch, E., Fong, W.T., et al., 2002. A comprehensive collection of chicken cDNAs. *Curr. Biol.* 12, 1965–1969.
- Bungo, T., Shimojo, M., Masuda, Y., Tachibanab, T., Tanaka, S.-J., Sugahara, K., et al., 1999. Intracerebroventricular administration of mouse leptin does not reduce food intake in the chicken. *Brain Res.* 817, 196–198.
- Chelikani, P.K., Glimm, D.R., Kennelly, J.J., 2003. Tissue distribution of leptin and leptin receptor mRNA in the bovine. *J. Dairy Sci.* 86, 2369–2372.
- Chen, H., Charlat, O., Tartaglia, L.A., Woolf, E.A., Weng, X., Ellis, S.J., et al., 1996. Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice. *Cell* 84, 491–495.
- Chua Jr, S.C., Koutras, I.K., Han, L., Liu, S.M., Kay, J., Young, S.J., et al., 1997. Fine structure of the murine leptin receptor gene: splice site suppression is required to form two alternatively spliced transcripts. *Genomics* 45, 264–270.
- Couturier, C., Jockers, R., 2003. Activation of the leptin receptor by a ligand-induced conformational change of constitutive receptor dimers. *J. Biol. Chem.* 278, 26 604–26 611.
- Denbow, D.M., Meade, S., Robertson, A., McMurtry, J.P., Richards, M., Ashwell, C., 2000. Leptin-induced decrease in food intake in chickens. *Physiol. Behav.* 69, 359–362.
- Dridi, S., Raver, N., Gussakovsky, E.E., Derouet, M., Picard, M., Gertler, A., et al., 2000. Biological activities of recombinant chicken leptin C4S analog compared with unmodified leptins. *Am. J. Physiol.* 279, E116–E123.
- Dunn, I.C., Boswell, T., Friedman-Einat, M., Eshdat, Y., Burt, D.W., Paton, I.R., 2000. Mapping of the leptin receptor gene (LEPR) to chicken chromosome 8. *Anim. Genet.* 31, 290.
- Dyer, C.J., Simmons, J.M., Matteri, R.L., Keisler, D.H., 1997. Leptin receptor mRNA is expressed in ewe anterior pituitary and adipose tissues and is differentially expressed in hypothalamic regions of well-fed and feed-restricted ewes. *Domest. Anim. Endocrinol.* 14, 119–128.
- Fong, T.M., Huang, R.-R.C., Tota, M.R., Mao, C., Smith, T., Varnerin, J., et al., 1998. Localization of leptin binding domain in the leptin receptor. *Mol. Pharmacol.* 53, 234–240.
- Friedman-Einat, M., Boswell, T., Horev, G., Grishvarma, G., Dunn, I.C., Talbot, R.T., et al., 1999. The chicken leptin gene: Has it been cloned? *Gen. Comp. Endocrinol.* 115, 354–363.
- Friedman, J.M., 2002. The function of leptin in nutrition, weight and physiology. *Nutr. Rev.* 60, S1–S14.
- Ghilardi, N., Skoda, R.C., 1997. The leptin receptor activates janus kinase 2 and signals for proliferation in a factor-dependent cell line. *Mol. Endocrinol.* 11, 393–399.
- Haniu, M., Arakawa, T., Bures, E.J., Young, Y., Hui, J.O., Rohde, M.F., et al., 1998. Human leptin receptor. Determination of disulfide structure and N-glycosylation sites of the extracellular domain. *J. Biol. Chem.* 273, 28 691–28 699.
- Hiroike, T., Higo, J., Jingami, H., Toh, H., 2000. Homology modeling of human leptin/leptin receptor complex. *Biochem. Biophys. Res. Comm.* 275, 154–158.
- Hoggard, N., Hunter, L., Lea, R.G., Trayhurn, P., Mercer, J.G., 2000. Ontogeny of the expression of leptin and its receptor in the murine fetus and placenta. *Br. J. Nutr.* 83, 317–326.
- Horev, G., Einat, P., Aharoni, T., Eshdat, Y., Friedman-Einat, M., 2000. Molecular cloning and properties of the chicken leptin-receptor (CLEPR) gene. *Mol. Cell. Endocrinol.* 162, 95–106.
- Houseknecht, K.L., Baile, C.A., Matteri, R.L., Spurlock, M.E., 1998. The biology of leptin: a review. *J. Anim. Sci.* 76, 1405–1420.
- Houseknecht, K.L., Portocarrero, C.P., 1998. Leptin and its receptors: regulators of whole-body energy homeostasis. *Domest. Anim. Endocrinol.* 15, 457–475.
- Huang, Y., Ying, K., Xie, Y., Zhou, Z., Wang, W., Tang, R., et al., 2001. Cloning and characterization of a novel human leptin receptor overlapping transcript-like 1 gene (LEPROTL1). *Biochim. Biophys. Acta* 1517, 327–331.
- Ingvarsen, K.L., Boisclair, Y.R., 2001. Leptin and the regulation of food intake, energy homeostasis and immunity with special focus on periparturient ruminants. *Domest. Anim. Endocrinol.* 21, 215–250.
- Lee, G.H., Proenca, R., Montez, J.M., Carroll, K.M., Darvishzadeh, J.G., Lee, J.I., et al., 1996. Abnormal splicing of the leptin receptor in diabetic mice. *Nature* 379, 632–635.
- Lin, J., Barb, C.R., Matteri, R.L., Kraeling, R.R., Chen, X.X., Meinersmann, R.J., et al., 2000. Long form leptin receptor mRNA expression in brain, pituitary and other tissues in the pig. *Domest. Anim. Endocrinol.* 19, 53–61.
- Lin, J., Barb, C.R., Kraeling, R.R., Rampacek, G.B., 2001. Developmental changes in the long form leptin receptor and related neuropeptide gene expression in the pig brain. *Biol. Reprod.* 64, 1614–1618.
- Lindell, K., Bennett, P.A., Itoh, Y., Robinson, I.C.A.F., Carlsson, L.M.S., Carlsson, B., 2001. Leptin receptor 5' untranslated regions in the rat: relative abundance, genomic organization and relation to putative response elements. *Mol. Cell. Endocrinol.* 172, 37–45.
- Ohkubo, T., Tanaka, M., Nakashima, K., 2000. Structure and tissue distribution of chicken leptin receptor (cOb-R) mRNA. *Biochim. Biophys. Acta* 1491, 303–308.
- Raver, N., Vardy, E., Livnah, O., Devos, R., Gertler, A., 2002. Comparison of R128Q mutations in human, ovine, and chicken leptins. *Gen. Comp. Endocrinol.* 126, 52–58.
- Reidy, S.P., Weber, J.-M., 2000. Leptin: an essential regulator of lipid metabolism. *Comp. Biochem. Physiol. A* 125, 285–297.
- Richards, M.P., Poch, S.M., 2002. Quantitative analysis of gene expression by reverse transcription polymerase chain reaction and capillary electrophoresis with laser-induced fluorescence detection. *Mol. Biotechnol.* 21, 19–37.
- Richards, M.P., 2003. Genetic regulation of feed intake and energy balance in poultry. *Poult. Sci.* 82, 907–916.
- Ruiz-Cortes, Z.T., Men, T., Palin, M.-F., Downey, B.R., Lacroix, D.A., Murphy, B.D., 2000. Porcine leptin receptor: Molecular structure and expression in the ovary. *Mol. Reprod. Dev.* 56, 465–474.
- Sadowski, Y., Raver, N., Gussakovsky, E.E., Shochat, S., Livnah, O., Rubinstein, M., et al., 2002. Subcloning, expression, purification and characterization of recombinant human leptin-binding domain (LBD). *J. Biol. Chem.* 277, 46 304–46 309.
- Taouis, M., Chen, J.-W., Daviaud, C., Dupont, J., Derouet, M., Simon, J., 1998. Cloning the chicken leptin gene. *Gene* 208, 239–242.

- Taouis, M., Dridi, S., Cassy, S., Benomar, Y., Raver, N., Rideau, N., et al., 2001. Chicken leptin: properties and actions. *Domest. Anim. Endocrinol.* 21, 319–327.
- Tartaglia, L.A., 1997. The leptin receptor. *J. Biol. Chem.* 272, 6093–6096.
- Udagawa, J., Hatta, T., Naora, H., Otani, H., 2000. Expression of the long form of leptin receptor (Ob-Rb) mRNA in the brain of mouse embryos and newborn mice. *Brain Res.* 868, 251–258.
- White, D.W., Kuropatwinski, K.K., Devos, R., Baumann, H., Tartaglia, L.A., 1997a. Leptin receptor signaling. Cytoplasmic domain mutational analysis and evidence for receptor homo-oligomerization. *J. Biol. Chem.* 272, 4065–4071.
- White, D.W., Wang, Y., Chua Jr, S.C., Morgenstern, J.P., Leibel, R.L., Baumann, H., et al., 1997b. Constitutive and impaired signaling of leptin receptors containing the Gln•Pro extracellular domain *fatty* mutation. *Proc. Natl. Acad. Sci. USA* 94, 10 657–10 662.